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Evaluation of exhaled breath condensate pH as a biomarker for COPD

William MacNee [a,*], Stephen I. Rennard [b], John F. Hunt [c], Lisa D. Edwards [d], Bruce E. Miller [e], Nicholas W. Locantore [d], Ruth Tal-Singer [e,f]

[a] *ELEGI Colt Research Labs, MRC Centre for Inflammation Research, The Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland, UK*
[b] *Pulmonary and Critical Care Medicine, Nebraska Medical Centre, Omaha, USA*
[c] *University of Virginia, Charlottesville, VA, USA*
[d] *GlaxoSmithKline, Research Triangle Park, NC, USA*
[e] *GlaxoSmithKline, King of Prussia, PA, USA*

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Summary
Introduction: We assessed the utility of EBC pH as a biomarker in COPD in a large cohort of well-characterised individuals with COPD and control subjects from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study. We also determined short term reproducibility and the response of EBC to oral prednisolone.
Methods: EBC was collected with R-Tubes™, using techniques for sampling and measurement that have been shown to be reproducible.
Results: EBC pH was lower in COPD ($n = 676$, 7.29 ± SD 0.60) and in smoking controls ($n = 31$, 7.18 ± 0.85), compared with non-smoking controls ($n = 50$, 7.59 ± 0.44, $p = 0.0008$ and 0.0033 respectively), but was not different between COPD and smoking controls. There was no relationship between EBC pH and disease severity, as assessed by the percent predicted FEV_1, nor with airway inflammation as assessed by sputum leukocyte counts. Treatment with 20 mg.day-1 prednisolone for 4 weeks did not change EBC pH.
Conclusion: EBC pH is lower in COPD than in healthy control non-smokers, but does not differentiate COPD from smokers without COPD, relate to disease severity or to airway inflammation, and does not respond to corticosteroids. EBC pH therefore does not appear to be a useful biomarker in COPD.

* Corresponding author. Tel.: +44 131 242 6583; fax: +44 131 242 6582.
E-mail address: w.macnee@ed.ac.uk (W. MacNee).
[f] On behalf of the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) investigators.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterised by progressive airflow limitation and chronic airway inflammation. Characterisation of COPD patients has largely relied on the measurement of airflow limitation, as assessed by the forced expiratory volume in 1 s (FEV_1), which has been used to assess the effects of therapeutic interventions in COPD clinical trials. There is a need for a validated biomarker of the airway inflammation in COPD, both to aid in characterisation of patients and also to assess the effects of therapeutic interventions, particularly anti-inflammatory treatments.

Exhaled breath condensate (EBC) has been used as a non-invasive technique to assess a range of biomarkers in a variety of lung diseases including COPD.[1] A large number of studies have shown significant disease-associated differences in the levels of diverse inflammatory biomarkers in EBC including cytokines and prostanoids.[2] However, these studies have generally been small in number and employed different methodologies, resulting in a lack of reproducibility of the findings, variations in the levels of measured parameters and consequently the data are difficult to interpret.[3]

Acidification of the airways, as assessed by EBC pH, is thought to reflect airway inflammation and EBC pH has been shown to be low in diverse respiratory diseases including asthma, COPD, chronic cough, bronchiectasis, and adult respiratory distress syndrome.[4–8]

Exhaled breath condensate pH has been reported to correlate with other indices of airway inflammation including sputum neutrophilia[4] and to be reduced further in exacerbations of COPD in one preliminary study,[9] but not in a more definitive study. EBC pH measurements are perhaps the most validated of all measurements performed on EBC with subject variability, collection, storage and assay system variations having been evaluated.[10–14] Normative data for EBC pH have been established in large populations of healthy subjects,[10] although there are still issues with standardisation of methodology and reproducibility of EBC pH.[11,12]

The validity of EBC pH as a biomarker in COPD patients has not been fully assessed, as most studies have made measurements in small numbers of subjects without proper characterisation of confounding factors, such as smoking status. We therefore assessed the reproducibility of EBC pH and the effect of smoking in healthy subjects (Study 1) and the utility of EBC pH as a potential biomarker in a large, well-characterised cohort of patients with COPD (Study 2). In addition we evaluated the sensitivity of EBC pH to anti-inflammatory treatment with oral corticosteroids (Study 3).

The results of these studies have been previously reported in abstract form.[15,16]

Methods

Study 1: reproducibility of EBC pH and effects of smoking

We assessed the reproducibility of EBC pH in healthy non-smokers ($n = 6$, 2F 4M; mean age 48.8 ± SD 5.9 years), current smokers ($n = 10$, 7F, 3M; mean age 49.8 ± 6.9 years), and healthy ex-smokers (not smoking for >12 months and at least 10 pack years smoking history, $n = 6$, 5F, 1M; mean age 57.3 ± 8.1 years) (GSK study number SB332235/030). Exhaled breath condensate samples were collected as described previously[14] using RTubes™ (Respiratory Research, Charlottesville, Virginia) at 0, 1, 3, 4, 6 and 8 h beginning at 8am on each study day on each of two visits. Lunch was provided before the 4 h collection. The effect of an acute smoking challenge (3 cigarettes) was also assessed in current smokers at two separate visits. Subjects attended the unit for a screening visit (Visit 1) within 21 days prior to the study start. Each non-smoking group attended for two further study visits, 5–10 days apart. Current smokers attended the clinic for four further study days. These four visits occurred as two sets of two consecutive days, 5–10 days apart. Smokers refrained from smoking from midnight the night before, and during the day on Visits 1 (screening visit), 2 and 4. To study the acute effects of smoking, current smokers were allowed to smoke three cigarettes for 30 min before collection of EBC on Visits 3 and 5.

Study 2: assessment of EBC pH in the ECLIPSE cohort

Exhaled breath condensate was obtained in the **E**valuation of **C**OPD **L**ongitudinally to **I**dentify **P**redictive **S**urrogate **E**ndpoints (ECLIPSE) cohort of patients (GSK Study number: SCO104960; clinicaltrials.gov Identifier NCT 00292552, appendix I). The aims of the ECLIPSE study and the study protocol have been previously described.[17] In brief, ECLIPSE is a multicentre, three year longitudinal prospective study to identify novel endpoints in COPD. Individuals aged 40–75 years were recruited to the study if they had a smoking history of ≥10 pack years, a post-bronchodilator ratio between forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) ≤0.7 and an FEV_1 % predicted <80%. Smoking (≥10 pack years) and non-smoking (<1 pack year) control subjects were enrolled if they were aged between 40 and 75 years and had normal lung function (post-bronchodilator FEV_1 >80% and FEV_1/FVC ratio >0.7) (Table 1). Individuals were excluded if they were known to have PiSZ ZZnul or nulnul alpha-1-antitrypsin deficiency or a respiratory disorder other than COPD. All subjects underwent standard spirometry following 180 μg (2 puffs) of salbutamol.[18] Reflux/heartburn were self-reported in the ATSDLD-78 questionnaire. All measurements were made in patients at a time of clinical stability (at least six weeks from the last exacerbation). EBC and induced sputum were collected at the baseline ECLIPSE visit as described below.

Study 3: effects of oral corticosteroids on EBC pH in individuals with COPD (GSK study number RES106087; Clinicaltrials.gov identifier NCT00379730)

In a study separate from ECLIPSE, current/former smokers aged 40–80 yrs with a post-salbutamol FEV_1 of 30–80% predicted and an FEV_1/FVC ratio of <0.7 ($n = 35$, Table 2) were recruited to study the effects of oral corticosteroids

Table 1 Demographics of the subjects with assessment of exhaled breath condensate (EBC) and the total ECLIPSE population (ECLIPSE). Data are presented as mean ± SD or *n* (%) unless otherwise indicated.

	COPD subjects		Smoker controls		Non-smoker controls	
	EBC ($n = 676$)	ECLIPSE ($n = 2164$)	EBC ($n = 31$)	ECLIPSE ($n = 337$)	EBC ($n = 50$)	ECLIPSE ($n = 245$)
Mean age, years	63 ± 7	63 ± 7	58 ± 9	55 ± 9	58 ± 9	54 ± 9
Male, *n* (%)	433 (64%)	1413 (65%)	20 (65%)	187 (56%)	17 (34%)	92 (37%)
Mean FEV $_1$, L[a]	1.37 ± 0.51	1.35 ± 0.52	3.30 ± 0.83	3.33 ± 0.77	3.15 ± 0.69	3.28 ± 0.79
Mean % predicted FEV_1, L[a]	49 ± 16	48 ± 16	110 ± 13	109 ± 12	117 ± 13	115 ± 14
Mean FEV_1,/FVC, %[a]	44 ± 12	45 ± 12	80 ± 6	79 ± 5	80 ± 5	81 ± 5
Current smoker, *n* (%)	261 (39%)	784 (36%)	10 (32%)	204 (61%)	0	0
Mean pack years	47 ± 28	49 ± 27	27 ± 15	32 ± 21	1[b]	0.2 ± 1.1

[a] Values are post-bronchodilator.
[b] One subject reported prior smoking history.

on EBC pH and other biomarkers.[19] We have recently reported the effect in this study of corticosteroid therapy on serum concentrations of surfactant protein D in the absence of changes in pre- and post-bronchodilator FEV_1.[19] Individuals were excluded if they had had an exacerbation of COPD requiring steroid or antibiotics in the month prior to the 28-day screening period or were taking oral or inhaled steroids for 14 days consecutively in the 6 months prior to screening. Individuals were randomised to receive either placebo or 20 mg prednisolone day-1 for 4 weeks; EBC was obtained at baseline and after four weeks treatment with placebo or prednisolone.

Exhaled breath condensate

EBC collection was carried out as described previously.[14,20] All subjects were asked to refrain from eating, drinking and smoking (except in those subjects in which the acute effects of smoking were studied) at least 3 h prior to collection. EBC samples were obtained prior to lung function assessments and in those subjects who underwent sputum induction prior to this procedure. EBC samples were collected during tidal breathing for 10 min, without nose clips, using the RTube™ (Respiratory Research Inc, Charlottesville, Virginia), aliquoted and stored at −70 °C according to the manufacturer's instruction (http://www.rtube.com/products/rtube/overview_htm).

A 200 μL aliquot of EBC was used for the pH assay. Measurement of pH was performed by a central laboratory (Respiratory Research Inc, Charlottesville, Virginia for the healthy subjects study; Cenetron Diagnostics, Austin, Texas for both COPD studies) after de-aeration by bubbling argon through the sample at 2 L/min while monitoring pH until the reading stabilised, usually after 8 min of de-aeration with argon as described previously.[14,21] Amylase was measured in EBC as an indicator of salivary contamination. Measurable amylase levels were found in only 3.7% of samples.

EBC samples were kept at −70 until all the baseline samples had been collected and other baseline data were available, and were therefore stored at −70 for less than 2 years. A previous study has confirmed the stability of EBC pH following long-term storage.[21]

Table 2 Summary of demographic baseline characteristics of the COPD patients randomised to receive placebo or oral corticosteroids in Study 3. Data are presented as mean ± SD or *n* (%) unless otherwise indicated. Subjects had EBC measured at least once during the study. FVC: forced vital capacity.

	Placebo	Prednisolone
Subjects, *n*	15	20
Age, yrs	61 ± 10	63 ± 11
Males	87%	85%
FEV_1 L[a]	1.55 ± 0.60	1.61 ± 0.64
FEV_1 % predicted[a]	53 ± 17	54 ± 15
FEV_1/FVC %[a]	50 ± 8	48 ± 12
FEV_1 reversibility %	10 ± 23	19 ± 12
Smoking history, pack-yrs	48 ± 24	48 ± 29
Current smokers	53%	40%
Salbutamol use	73%	90%
Ipratropium bromide use	33%	40%

[a] Post-bronchodilator.

Sputum induction

Sputum induction was performed on 398 subjects from the same subgroup of COPD patients in the ECLIPSE cohort (Study 2) who had EBC collected at the baseline visit. Sputum was induced using 3 × 7 minute inhalations of 3% saline. Sputum plugs were extracted and the samples were processed with dithiotreitol (DTT, 0.1% in distilled water). PBS was added and the samples were passed through a filter and centrifuged. Cytospins were prepared from the cell pellets, air dried, fixed with methanol and stained with Rapi-diff (Triangle, Skelmersdale, UK). Five hundred leukocytes were counted by two independent readers at a central laboratory (Dr Rennard's laboratory, University of Nebraska, USA) and the results expressed as a percentage of the total leucocyte count, and a total cell number/ml.

Statistical analysis

In the reproducibility study (Study 1), mixed effects models were fitted with subject as a random effect and subject

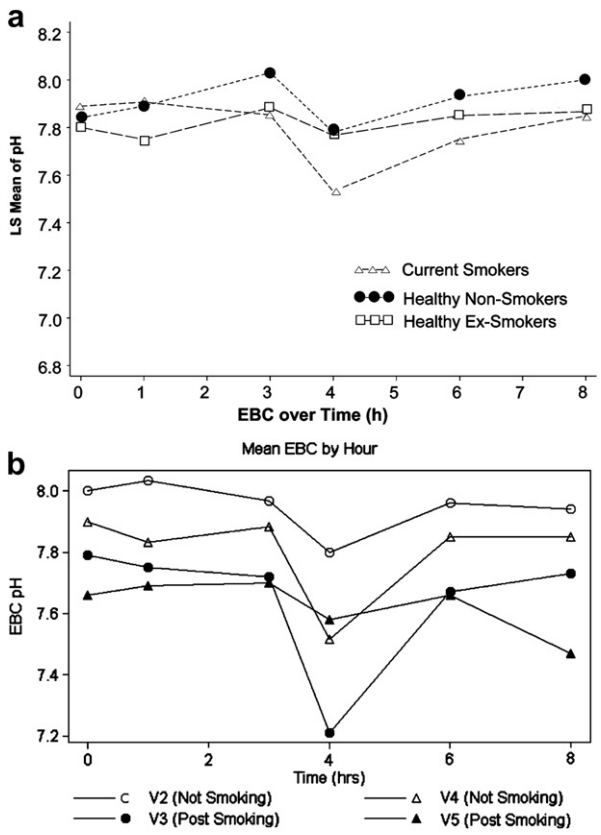

Figure 1 (a). Mean EBC pH over time by Subject Group (Study 1) healthy current, ex and non-smokers. Healthy current smokers had abstained from smoking for at least 8 h. There are no significant differences between the groups. (b). mean EBC pH over time in healthy current smokers at visits 2 and 4 when they had abstained from smoking prior to the study (not smoking) and at visits 3 and 5 when they had smoked 3 cigarettes for 30 min before the collection of EBC (post-smoking).

group, day and time as fixed effects, as needed. Comparisons of EBC pH between subject groups were obtained from the above models by constructing appropriate contrasts. To investigate the acute change in EBC pH and variability in response to cigarette smoke, a mixed effects model was fitted with visit and time as fixed effects and subject as a random effect. The model allowed for unequal variances pre- and post-smoking. The difference in the EBC pH when abstaining from smoking (ie, Visits 2 and 4) and post-smoking (ie, Visits 3 and 5) was calculated by constructing the appropriate contrast.

In study 2, sample sizes of 676 COPD subjects and 31 smoker controls (50 non-smoker controls) provided 89% (97%) power to detect a difference of 0.5 between the two groups based on a two-sided *T*-test with $\alpha = 0.05$ and an assumed standard deviation of 0.85. Statistical analysis in the ECLIPSE cohort (Study 2) was performed by ANOVA and pairwise comparisons.

The Shapiro—Wilk test indicated that the distribution of pH was non-normal. Thus Spearman correlation coefficients were calculated to investigate the relationship between EBC pH and induced sputum cell counts.

In the prednisolone study (Study 3), the effect of prednisolone on EBC pH and FEV_1 was analysed by ANOVA, adjusting for baseline value and study site.

Ethics

The studies were all conducted in accordance with the declaration of Helsinki and ICH Good Clinical Practice guidelines and were approved by the relevant Ethics and Institutional Review Boards at the participating centres.

Results

Study 1: reproducibility of EBC pH and effects of smoking

Exhaled breath condensate pH was highly reproducible in almost all subjects (CV range 1—14%), both within a given day and on separate visits (Fig. 1a and Table 3). However, a small number of subjects revealed variability on one or more visits, usually post-prandially at the 4 h time point (Fig. 1a), a trend that persisted at the 6 h collection in some subjects. The within-subject variance of pH was not affected by subjects being allowed to smoke (0.0721 when abstained from smoking, compared to 0.0750 when allowed to smoke).

When EBC pH was compared across groups of subjects who were not allowed to smoke, no statistically significant differences were observed (data not shown). In the current smoker population, EBC pH was significantly higher when subjects abstained from smoking compared with post-

Table 3 Within and between subject variability in EBC pH (Study 1).

Assessment day	Group	Variability estimates	
		Within-subject	Between-subject
Days 1 and 2	Healthy non-smokers	0.0444	0.0444
	Current smokers	0.0774	00962
	Healthy ex-smokers	0.0382	0.0475
Day 1 only	Healthy non-smokers	0.0265	0.0346
	Current smokers	0.1122	0.1543
	Healthy ex-smokers	0.0303	0.0441
Day 2 only	Healthy non-smokers	0.0386	0.0492
	Current smokers	0.0401	0.0457
	Healthy ex-smokers	0.0096	0.0256

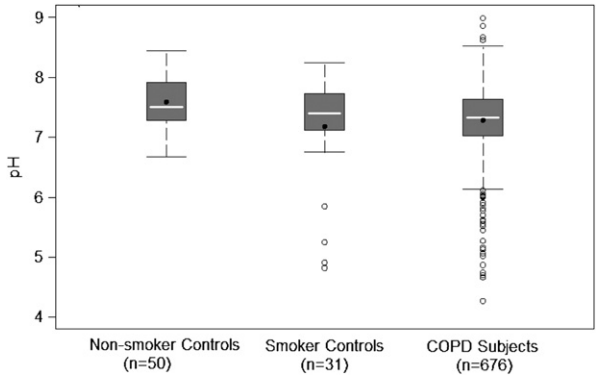

Figure 2 EBC pH values in non-smoker and smoker controls and COPD patients. Vertical bars represent median and inter-quartile range (●: mean; ○: outliers). Mean EBC pH was lower in patients with COPD, compared with non-smoker controls ($p = 0.0008$) and in smoker controls versus non-smoker controls ($p = 0.0033$). The difference between COPD patients and smoker controls was not significant ($p = 0.33$).

smoking EBC pH (mean treatment difference: 0.16; 95% CI: 0.01, 0.31). Mean EBC pH values are graphically represented in Fig. 1b.

In the current smoker population, excluding those with a positive carbon monoxide breath test (exhaled breath carbon monoxide level of ≥10 ppm) at baseline, results were similar to those obtained with all current smokers included in the analysis (treatment difference 0.24; 95% CI: 0.10, 0.38).

Study 2: assessment of EBC pH in the ECLIPSE cohort

Exhaled breath condensate pH was measured in samples from 757 individuals (676 COPD patients, 31 smoker controls and 50 non-smoker controls). The demographics of the exhaled breath cohort were similar to those of the entire population of subjects enrolled in the ECLIPSE study, with the exception that in the smoking controls there were less current smokers and more males in the EBC cohort than in the full ECLIPSE cohort (Table 1).

A significantly lower mean EBC pH was observed in patients with COPD, compared with non-smoker controls ($p = 0.0008$) and in smoker controls versus non-smoker controls ($p = 0.0033$). The difference between COPD patients and smoker controls was not significant ($p = 0.33$) (Fig. 2).

When COPD patients and smoker control subjects were divided according to smoking status (current versus former smokers), EBC pH was significantly lower in all smoker groups compared to non-smoker controls. There was, however, no difference between COPD, whether current or ex-smokers, and smoker controls (Fig. 3a). When COPD subjects were categorised according to GOLD stage,[22] there was no relationship between EBC pH and disease severity as assessed by the FEV_1 % predicted (Fig. 3b).

Stratification of the COPD subjects and controls according to the presence or absence of gastro oesophageal reflux showed no significant differences in EBC pH. Nor were there any significant differences in EBC pH between patients who did or did not receive treatment with inhaled corticosteroids or long acting beta agonists.

A subgroup of 389 subjects had both EBC and sputum sampled and had demographics which were not different from the ECLIPSE cohort as a whole (data not shown). In this group there was no relationship between EBC pH and airway inflammation as assessed by sputum leukocyte counts (eosinophils, lymphocytes or neutrophils, $r = 0.052$, 0.027, 0.080, respectively, $p > 0.05$).

Study 3: effects of oral corticosteroids on EBC pH in individuals with COPD

A total of 35 current or former smokers diagnosed with COPD were recruited and randomised to receive either oral prednisolone or placebo for four weeks. The groups were well matched for age, sex, lung function and smoking history (Table 2). At baseline EBC pH values were lower in the placebo group than in the prednisolone treated group, possibly as a result of a higher percentage of current smokers than in the prednisolone group(53% vs 40%), which could have resulted in lower EBC pH values.

There were three withdrawals in the prednisolone group and two in the placebo group during the course of the study. Treatment with prednisolone resulted in a small increase relative to placebo in pre- and post-bronchodilator FEV_1 (compared to trough) of 41 and 74 mL, respectively; neither of these changes were statistically significant ($p > 0.5$ for both). There was no change in EBC pH after treatment with prednisolone (Fig. 4).

Discussion

Exhaled breath condensate (EBC) is a method of sampling the air from the airways non-invasively that is easily repeated and acceptable to patients. As the expired air is cooled, water vapour condenses and then traps volatile substances evolving from the airway lining fluid. When the airway lining source fluid is acidic, there is more proton-ation of anions into uncharged—and volatile—acids, which are then more readily exhaled and captured, reducing the pH of the EBC. Guidelines on the use of EBC have been published by the European Respiratory Society Task Force.[23]

In assessing the potential of EBC pH as a biomarker in COPD the following ideal characteristics of a pulmonary biomarker should be assessed 1) reproducibility; 2) disease specificity; 3) the ability of biomarkers to differentiate between health and disease; 4) the ability of the biomarker to distinguish different severities and phenotypes of disease, and 5) the ability of the biomarker to change following an intervention.

Acidification of the airways is thought to reflect airway inflammation. A study of >400 healthy subjects has defined an "normal range" for EBC pH where the median pH was 8.0[10] In Study 1, EBC pH was highly reproducible in almost all subjects, both within a given day and on separate visits, in accordance with reported evidence of EBC pH repro-ducibility.[14] There was a post-prandial reduction in EBC pH in several individuals (possibly due to gastro-oesophageal reflux or ingestion of acidifying foods). Thus in subsequent

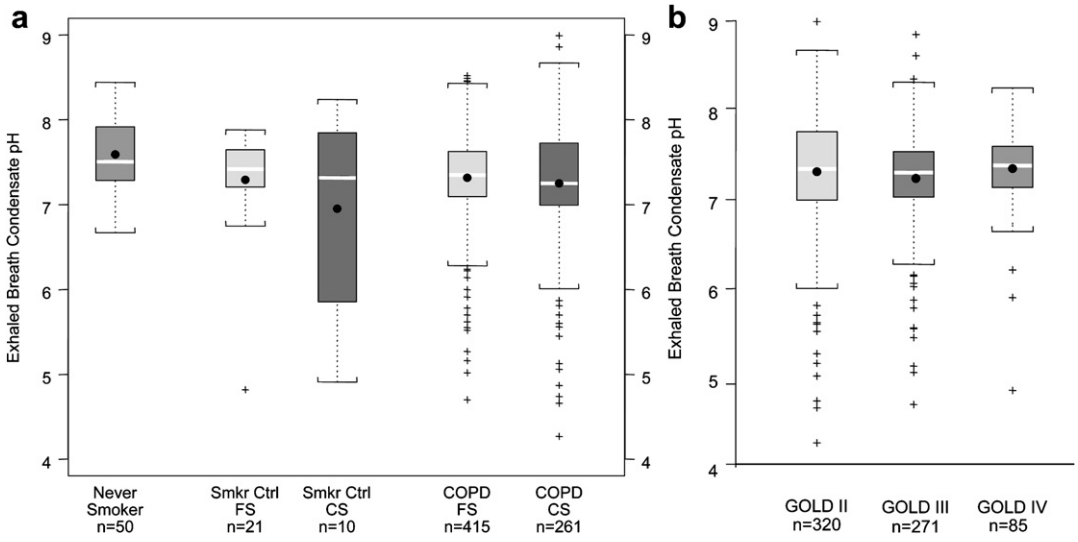

Figure 3 EBC pH values in COPD and controls according to (a) smoking history. EBC pH was significantly lower in all smoker groups compared to non-smoker controls ($p \leq 0.028$), but no difference between COPD, whether current or ex-smokers and smoker controls. (b) EBC pH values in COPD according to disease severity as assessed by GOLD stage. There are no significant differences between the groups. Vertical bars represent median and interquartile range (●: mean; +: outliers). Smkr ctrl = smoker control subject; FS = former smoker; CS = current smoker.

studies we did not collect EBC for at least 2 h after consuming anything other than water. There was an acute effect of smoking on EBC pH. Thus in subsequent studies we asked subjects to abstain from smoking for at least 3 h prior to EBC collection to reduce assay variability due to the acute effects of smoking.

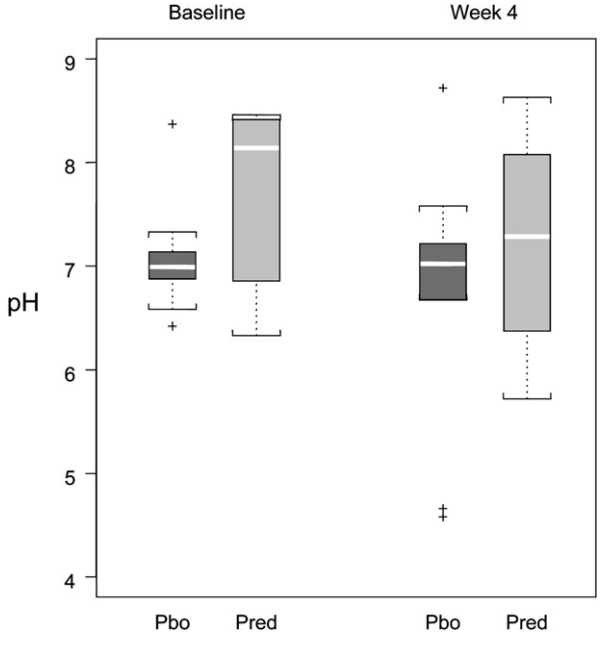

Figure 4 Effects of placebo or prednisolone on EBC pH in COPD patients. Vertical bars represent the median and interquartile range (+: outliers) and the horizontal lines the medians. There are no significant changes in EBC pH after prednisolone (Pred) or placebo (Pbo).

The factors that influence the reproducibility of EBC pH have been studied previously in COPD patients.[11] These studies showed that COPD patients have a higher within and between day variability in EBC pH than normal subjects and that measurements change over time in COPD patients for reasons which are not clear, but may relate to several factors, such as airway inflammation, oxidative stress, gastroesophogeal reflux and bacterial colonisation, which are known to influence EBC pH.[14]

We used the established technique which employed Argon de-aeration to remove all volatile components of EBC allowing the measurement of non volatile acidity. More recent studies have suggested that this technique may not remove CO_2 which may influence the value of EBC pH and that measurement of pH at a standard CO_2 partial pressure may improve the reproducibility of the measurement.[24] However we were able to establish good reproducibility of pH measurements using the technique which we employed.

In Study 2, we have confirmed the results of previous studies in smaller groups of patients[4,11] that patients with COPD have reduced EBC pH. However, there is considerable overlap between COPD subjects and normal non-smokers. This contrasts with previous studies which have suggested a clear differentiation between the EBC pH normal range and the EBC pH observed in COPD patients.[4] An important strength of Study 2 is that the relatively large sample size provides a much better indication of the true distribution of EBC pH than is possible in smaller studies. Previous studies have shown marked overlap between the results obtained in COPD and other airways diseases such as asthma[4,20] and cystic fibrosis[25] and have also not taken into account confounding factors in COPD patients, such as the effects of cigarette smoking. We also found that cigarette smokers without COPD also had acidification in their airways with lower EBC pH than healthy normal subjects, but found no difference in EBC pH between smoker controls and COPD

subjects. Furthermore, when subjects were separated according to their smoking status (current versus former smokers), EBC pH was significantly lower in all smoking groups compared to non-smoking controls. There was no difference between COPD patients divided by smoking status and smoker controls; however ex-smokers with COPD did retain lower EBC pH than healthy non-smokers.

We studied the influence of the presence of gastro oesophageal reflux on EBC pH and found no differences in EBC pH in those with or without reported gastro oesophageal reflux. In addition treatment with inhaled corticosteroids or long acting beta agonists did not appear to influence EBC pH.

A previous study in a small group of patients has shown a relationship between EBC pH and disease severity as measured by the FEV_1,[4] although this was not confirmed in another COPD population.[11] In Study 2, in a large group of COPD patients, no relationship between EBC pH and disease severity as measured by the FEV_1 was found.

Exhaled breath condensate is thought to reflect airway inflammation and, indeed, in a small study a relationship was shown between EBC pH and sputum neutrophils.[4] However in Study 2, in a large group of COPD patients, we were unable to find any relationship between airway inflammation as assessed by sputum leukocyte counts and EBC pH. In a previous cross sectional study in COPD patients EBC pH was not different whether patients were treated or not with inhaled corticosteroids.[4] We were also unable to show an effect of the anti-inflammatory agent prednisolone on EBC pH (Study 3). This contrasts with a previous study in the same patients in which prednisolone reduced the levels of surfactant protein-D, a marker of airway inflammation/injury.[19]

In summary, EBC pH distinguished smoker controls and COPD patients from non-smoker controls, but did not distinguish smoker controls from COPD patients. We found no differences in EBC pH related to disease severity, nor a relationship between EBC pH and airway inflammation as assessed by sputum leukocyte counts. EBC pH was not affected by treatment with an oral corticosteroid. When taken together, these data suggest that EBC pH does not distinguish COPD subjects from current/former smokers with normal lung function and is not useful as a biomarker for assessment of disease severity or the effect of interventions in COPD.

Acknowledgement

We would like to express our thanks to all subjects who took part in these studies. All three studies were funded by GlaxoSmithKline.

Statement of interest

Professor William MacNee: WMcN has been reimbursed for travel by GlaxoSmithKline, AstraZeneca, Boehringer Ingelheim, Pfizer and Micromet for attending conferences. WMcN has received honoraria from GlaxoSmithKline and AstraZeneca for participating as a speaker in scientific meetings. WMcN serves on advisory boards for GlaxoSmithKline, Pfizer, Almirall, Amgen, Bayer and Micromet. WMcN serves as a consultant for Pfizer and SMB Pharmaceuticals. Research grants to support work carried out in WMcN's laboratory comes from SMB, Pfizer, GlaxoSmithKline and Novartis.

Stephen I. Rennard: StIR has had or currently has a number of relationships with companies who provide products and/or services relevant to outpatient management of chronic obstructive pulmonary disease. These relationships include serving as a consultant, advising regarding clinical trials, speaking at continuing medical education programs and performing funded research both at basic and clinical levels. StIR does not own any stock in any pharmaceutical companies.

Specific companies with whom StIR have had relationships within the last three years include:

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Dey	Schering-Plough
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Novartis	
Lecture Fees	
ACCP	Network for Continuing Ed
AstraZeneca	Novartis
Creative Educational Concept	Pfizer
France Foundation	SOMA
Industry-Sponsored Grants	
Almirall	Lorillard
AstraZeneca	Novartis
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GlaxoSmithKline	Roche
IFSH	

Please note that StIR has had tobacco industry funding. Specifically, StIR has received funding from the tobacco industry for studies relating to harm reduction and to the impact of tobacco smoke on stem cells. StIR has also consulted with RJ Reynolds without personal fee on the topic of harm reduction. StIR received funding from RJ Reynolds to evaluate the effect of a harm reduction product in normal smokers (1996) and in subjects with chronic bronchitis (1999) and to assess the effect of smoking cessation on lower respiratory tract inflammation (2000); StIR participated in a Philip Morris multi-center study to assess biomarkers of smoke exposure (2002); StIR received funding for a clinical trial from the Institute for Science and Health (2005), which receives support from the tobacco industry, to evaluate biomarkers in exhaled breath associated with smoking cessation and reduction. This study was supplemented with funding from Lorillard and RJ Reynolds. StIR has received a grant from the Philip Morris External Research Program (2005) to assess the impact of cigarette smoking on circulating stem cells in the mouse. StIR has consulted with RJ Reynolds on the topic of harm reduction until 2007, but did not receive personal remuneration for this. There are no active tobacco-industry funded projects. All ties with tobacco industry companies and entities supported by tobacco companies were terminated in 2007.

John Hunt, MD: JH is a co-founder of Respiratory Research, Inc., which develops exhaled breath assays and manufactures the RTubes used in this study. Respiratory Research has also provided funding for his laboratory. His University of Virginia research lab receives funding indirectly from Philip Morris through the University of Virginia Phillip Morris Tobacco Research Program, over which Phillip Morris has no supervision authority.

Lisa D Edwards: LDE is an employee of GlaxoSmithKline; holds stocks and stock options in GlaxoSmithKline.

Bruce E. Miller: BEM is an employee of GlaxoSmithKline and owns GSK stock and stock options.

Nicholas Locantore: NL is an employee of GlaxoSmithKline, and also holds GlaxoSmithKline stock.

Ruth Tal-Singer: RT-S is an employee and shareholder of GlaxoSmithKline, the sponsor of the three studies.

Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rmed.2011.02.009.

References

1. Borrill ZL, Roy K, Singh D. Exhaled breath condensate biomarkers in COPD. *Eur Respir J* 2008;**32**(2):472—86.
2. Kharitonov SA, Barnes PJ. Exhaled biomarkers. *Chest* 2006; **130**(5):1541—6.
3. Koutsokera A, Loukides S, Gourgoulianis KI, Kostikas K. Biomarkers in the exhaled breath condensate of healthy adults: mapping the path towards reference values. *Curr Med Chem* 2008;**15**(6):620—30.
4. Kostikas K, Papatheodorou G, Ganas K, Psathakis K, Panagou P, Loukides S. pH in expired breath condensate of patients with inflammatory airway diseases. *Am J Respir Crit Care Med* 2002; **165**(10):1364—70.
5. Carraro S, Folesani G, Corradi M, Zanconato S, Gaston B, Baraldi E. Acid-base equilibrium in exhaled breath condensate of allergic asthmatic children. *Allergy* 2005;**60**(4):476—81.
6. Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. *Thorax* 2005;**60**(1):22—6.
7. Niimi A, Nguyen LT, Usmani O, Mann B, Chung KF. Reduced pH and chloride levels in exhaled breath condensate of patients with chronic cough. *Thorax* 2004;**59**(7):608—12.
8. Gessner C, Hammerschmidt S, Kuhn H, Seyfarth HJ, Sack U, Engelmann L, et al. Exhaled breath condensate acidification in acute lung injury. *Respir Med* 2003;**97**(11):1188—94.
9. Antus B, Barta I, Kullmann T, Lazar Z, Valyon M, Horvath I, Csiszer E. Assessment of exhaled breath condensate pH in exacerbations of asthma and chronic obstructive pulmonary disease A longitudinal study. *Am J Respir Crit Care Med* 2010; **182**:1492—7.
10. Paget-Brown AO, Ngamtrakulpanit L, Smith A, Bunyan D, Hom S, Nguyen A, et al. Normative data for pH of exhaled breath condensate. *Chest* 2006;**129**(2):426—30.
11. Borrill Z, Starkey C, Vestbo J, Singh D. Reproducibility of exhaled breath condensate pH in chronic obstructive pulmonary disease. *Eur Respir J* 2005;**25**(2):269—74.
12. Effros RM, Casaburi R, Su J, Dunning M, Torday J, Biller J, et al. The effects of volatile salivary acids and bases on exhaled breath condensate pH. *Am J Respir Crit Care Med* 2006;**173**(4): 386—92.
13. McCafferty JB, Bradshaw TA, Tate S, Greening AP, Innes JA. Effects of breathing pattern and inspired air conditions on breath condensate volume, pH, nitrite, and protein concentrations. *Thorax* 2004;**59**(8):694—8.
14. Vaughan J, Ngamtrakulpanit L, Pajewski TN, Turner R, Nguyen TA, Smith A, et al. Exhaled breath condensate pH is a robust and reproducible assay of airway acidity. *Eur Respir J* 2003;**22**(6):889—94.
15. Tal-Singer R, Simpson KJ, Vaughan J, Linacre P, Gibson A, Ngamtrakulpanit L, Anselm RS, Vessey SJ, Hunt J. Reproducibility of exhaled breath condensate pH in healthy subjects (smokers and non-smokers). *Am J Respir Crit Care Med* 2004; **169**:A825.
16. MacNee W, Rennard SI, Edwards LD, Miller BE, Tal-Singer R. *Utility of exhaled breath condensate pH as a biomarker in COPD remains undefined*. ERS Congress; 2008. e-communication E3117.
17. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, et al. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir J* 2008; **31**(4):869—73.
18. American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995;**152**(3):1107—36.
19. Lomas DA, Silverman EK, Edwards LD, Locantore NW, Miller BE, Horstman DH, Tal-Singer R. Serum surfactant protein D is steroid sensitive and associated with exacerbations of COPD. *R.Eur Respir J* 2009;**34**:95—102.
20. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, et al. Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Respir Crit Care Med* 2000;**161**(3 Pt 1): 694—9.
21. Vaughan J, Ngamtrakulpanit L, Pajewski TN, Turner R, Nguyen TA, Smith A, Urban P, Hom S, Gaston B, Hunt J. Exhaled breath condensate pH is a robust and reproducible assay of airway acidity. *Eur Respir J* 2003;**22**:889—94.
22. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and

prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;**176**(6): 532—55.
23. Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005;**26**(3): 523—48.
24. Kullmann T, Barta I, Lazar Z, Szili B, Barat E, Valyon M, Kollai M, Horvath I. Exhaled breath condensate pH standardised for CO_2 partial pressure. *Eur Respir J* 2007;**29**:496—501.
25. Carpagnano GE, Barnes PJ, Francis J, Wilson N, Bush A, Kharitonov SA. Breath condensate pH in children with cystic fibrosis and asthma: a new non-invasive marker of airway inflammation? *Chest* 2004;**125**(6):2005—10.